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TRITIUM ACCUMULATION IN LETTUCE FUMIGATED WITH ELEMENTAL TRITIUM

J. C. McFarlane

Environmental Monitoring and Support Laboratory
Las Vegas, Nevada

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16. ABSTRACT

Lettuce plants fumigated with elemental tritium accumulated tritium in the plant water as well as in the organic constituents. The conversion rate of elemental tritium to tritiated water varied from 0.5 nanocuries per minute per pot at the start to 0.8 nanocuries per minute per pot at the termination of the 24-day exposure to an air concentration of 5 nanocuries per liter. Based on the concentration of tritium in various plant tissues, foliar absorption was postulated as the route of plant contamination. The data indicated that an enzymatically facilitated conversion on the leaf or soil surface was the probable mechanism.

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by

J. C. McFarlane
Monitoring Systems Research and Development Division
Environmental Monitoring and Support Laboratory
Las Vegas, Nevada 89114

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INTRODUCTION

In the past, tritium was a pollutant associated primarily with the testing of nuclear weapons. In the decade prior to the Test Ban Treaty of 1963, approximately 1,700 megacuries of tritium were injected into the environment through such activities (Weaver et al. 1969). This release of large quantities of tritium into the world environment increased the natural equilibrium concentrations by a factor of 25. Due to radioactive decay, the present concentration will decrease until about 1995 when a new increase is predicted to occur because of increasing releases of tritium from the nuclear power industry (Cowser, et al., 1966). In the production of electricity by nuclear reactors, approximately 50 mCi of tritium are produced per day for each megawatt of electrical energy generated. Most of the tritium produced is retained within the fuel element and released during reprocessing. It has been estimated that about 25% of the tritium produced is released by the stack, 65% as liquid, and only 10% is retained in waste storage (Weaver, et al., 1969). Evaluations by Cochran et al. (1973) indicate that approximately 25% of the tritium released through the stack was in a gaseous state (T_2) and 75% as water vapor. The Radiological Science Laboratory in the New York State Department of Health (USEPA, 1972) has preliminary information which they report indicates that possibly 90% of the tritium released from nuclear fuel reprocessing is elemental and has not been previously accounted for by routine monitoring of HTO. Recent advances in the sampling of non-aqueous tritium, allow a more accurate determination of gaseous tritium releases from various phases of the nuclear power industry (Griffin et al. 1973).

The importance of gaseous tritium (HT or T_2) as an aerial pollutant has largely been discounted because of the relative stability of hydrogen gas. It was shown by Pinson (1951) that although oxidation or exchange of HT occurred during inhalation, less than 0.004%

of the inhaled dose was retained by man. When bean leaves were exposed to HT in air at 200 mCi/cc, the extractable tritium (HTO) reached levels up to 11,000 mCi/g of plant tissue (Cline, 1953).

The guidelines for occupational exposure to elemental tritium (ICRP publication No. 2, 1959) are based on a decreased absorption in man and consequently lower doses than when exposed to similar concentrations of HTO. The maximum permissible concentrations of tritium gas (HT) and tritiated water (HTO) in air, are 400 pCi/cc and 5 pCi/cc, respectively. Eakins and Hutchinson (1973) reported that the conversion of HT to HTO was a very slow reactions. They calculated the conversion times for these reactions in the presence of various catalyzing surfaces. Reaction half-times varied from four years in the presence of platinum to 1151 years for glass. Based on these findings they suggest that elemental tritium was of no consequence as a local contaminant.

The uncertainty of gaseous tritium release and the lack of a critical evaluation regarding the influence of plants and soils on the oxidation or exchange of HT to HTO make it important that additional information be obtained. If large amounts of tritium are released in the reprocessing of nuclear fuels, it is important to know the environmental impact of this pollutant. The research discussed in this report was aimed at determining the importance of tritium as a gas in the contamination of plants and soils.

METHODS

Lettuce plants (Lactuca sativa var. Grand Rapids) were grown in a mixture of peat and vermiculite (1:1) and watered with a modified Hoaglands nutrient solution (Berry 1971). Water was applied at 4-hour intervals and sufficiently over-watered (100 ml per application) at each period to cause drainage through the pots. This ensures optimum water availability and constant nutrient availability.

Plants were grown from seeds in 5-inch pots and 25 plants of uniform size were transferred from the glasshouse to the growth chamber after 12 days. Treatment was started 24 days after seeding at which time the foliar fresh weight was about 12 g per plant and the 11th node was just developing.

Studies were conducted in plant growth chambers similar to those designed by Hill (1967). Light was produced by cool white/fluorescent 1500-milliamp tubes and 200-watt incandescent lamps (input wattage: 73% and 27%, respectively). The radiant flux was measured between 450 and 950 nanometers in the center of the chamber at pot height; it decreased from 220 to 200 watts per square meter from the beginning until the termination of the experiment. Light exposure was 16 hours with an abrupt light/dark change. The air temperature at the top of the plants was 25 $\pm$ 0.5°C during the light period and 20 $\pm$ 0.5°C during the dark. The relative humidity was maintained at 75 $\pm$ 5% and the air flow moved horizontally across the chamber at approximately 50 m/minute. The growth chamber is an air tight system and therefore required the injection of carbon dioxide to maintain photosynthesis. The injection rate of CO_2 required to maintain a constant concentration (350 parts per million) was done automatically. The recorded flow rate gave a measure of the CO_2 assimilation rate. Humidity in the chamber was controlled by the temperature of a cold-water condensation coil. The condensate from this coil was collected in a fraction collector. The times of collecting each fraction were recorded and used to calculate the rate of transpiration. Leachate from the pots was collected through a common drain in the bottom of the chamber.

Elemental tritium was received in a stainless steel bottle pressurized with N_2. The absence of HTO was verified by sampling a dilute standard gas directly as well as through a column of anhydrous $CaSO_4$ (the resultant dew point was < -5°C). No variation occurred

between the two samples, thus indicating the absence of HTO as an impurity. Tritium concentration was determined by a flow-through internal proportional tritium analyzer (Johnson Laboratories Inc., Tritium Monitor Model 112 B). The air to be analyzed was drawn at the rate of 5 liters per hour through a column of solid desiccant was subsequently removed and shown to have a tritium concentration (HTO) equivalent to that found in the chamber atmospheric moisture collected by alternate means.

The output from the tritium analyzer was connected to a limit switch which activated a valve when the concentration decreased below the desired level. Upon demand, a timed, metered pulse of tritium from the pressurized bottle was injected into the chamber. The flow rate and duration of this pulse were recorded on a strip chart recorder and used to calculate the amount of elemental tritium required to maintain the prescribed concentration (5 nCi/l). Since the chamber was a closed system, any decrease in tritium gas concentration must have been caused by a conversion of HT/T_2 to HTO. By integrating the injection rate over time, the HT to HTO conversion rate was calculated. This automatic system allowed the simulation of a uniform HT contaminated environment throughout the test period.

Five plants were randomly selected from the plants remaining in the chamber on days 7, 12, 18 and 24. On day 18, five young plants and five unplanted pots were placed in the chamber and were sampled on day 24. The outer 16 leaves of each plant were separated from the stem and constituted the "outer" or old leaf tissue. Most of these leaves were completely expanded and none were smaller than 2 cm long. The remaining leaves constituted the inner or new tissue. Roots were separated from the potting mix by shaking.

Samples collected for water analysis were placed immediately into a 1-liter round-bottom flask containing benzene. The criticality of appropriate plant sampling is discussed by McFarlane *et al.* (1975)

and all their precautions were used. Plant and soil water was extracted via azeotropic distillation as reported by Moghissi et al. (1973).

The tritium concentrations associated with the organic constituents of the plants were determined by oxidizing the dry plant material in a pressurized oxygen bomb and subsequently analyzing the water of combustion (Moghissi, et al., 1975). Tritium concentrations in leachate, transpired water, water extracted from the plants, and the water of combustion, were analyzed using liquid scintillation techniques as described by Lieberman and Moghissi (1970).

RESULTS AND DISCUSSION

Tritium concentrations in leaf water and organic plant material are shown in Figure 1. Equilibrium in the plant free water occurred at about day 12. The tritium concentration in the organic fraction increased throughout the exposure. The organic tritium concentration in the outer or older leaves was slightly lower throughout the experiment. However, organic tritium increases in the older, middle and younger tissues were similar; this indicates that all tissues were growing and actively producing and utilizing metabolites. By day 24, approximate equilibrium was obtained. The different slopes of tritiated free water and tritium in the organic phase can be explained on the basis of the organic plant material already in existence before the tritium fumigation started. During the last 2 weeks of fumigation, plant growth had reached its maximum rate. In this latter stage, the dilution caused by uncontaminated organic material was insignificant and the concentration found is thought to represent the equilibrium level.

Tritium concentration in the lettuce roots is shown in Figure 2. The organic tritium concentration was constant after day 12. This value was approximately 25% of the organic tritium concentration

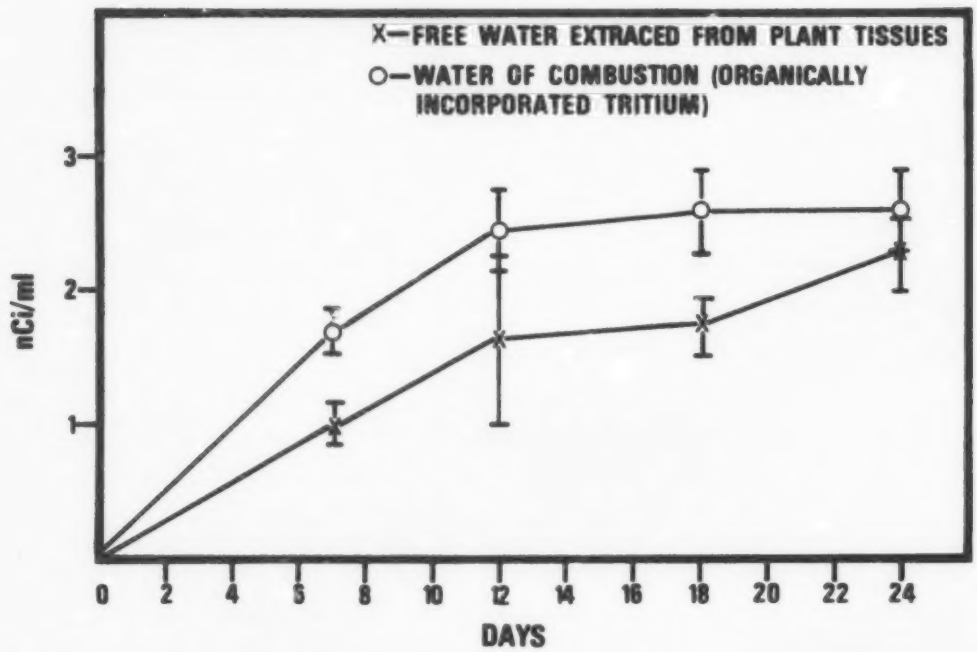

Figure 1. Tritium concentration in lettuce leaves exposed to 5 nCi/l of HT

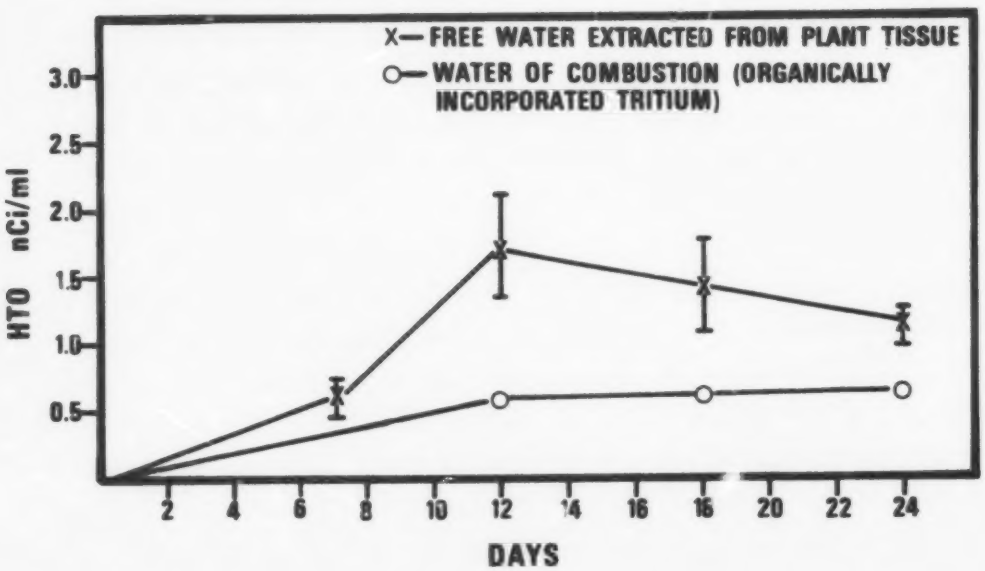

Figure 2. Tritium concentration in lettuce roots of plants exposed to 5 nCi/l of HT

in the aerial plant organs. Free water tritium reached a maximum of about 68% of the concentration in the leaves. The decrease in extractable HTO after day 12 is thought to be caused by higher transpiration rates and the development of the rooting system deeper in the pot. The concentration gradient of tritium in both free water and organic material from leaves to roots suggests that the route of contamination was foliar. The gradient between tritium in the organic fractions of leaves and roots could indicate that organic material synthesized in the leaves and translocated to the roots was subject to exchange with hydrogen or some hydrolytic activity.

At the termination of the tritium fumigation, a set of samples was transferred to another growth chamber to determine the rate of tritium loss. It was found that the tritium concentration in the free water fraction decreased exponentially and that the loss rate was independent of the age of the leaf sample (Figure 3). By day 3, the extractable water from the leaves contained less than 10% of the original concentration. An initial decrease of approximately 30% occurred in all organic tritium (Figure 4). This was probably the result of protium exchange with tritium on the hydroxyl units of the cellulose (Lang and Mason, 1960). After this initial decrease, the tritium concentration in the organic fraction of the inner leaves decreased more rapidly than the concentration in the outer leaves. This can be explained by the dilution created by the synthesis of new untritiated plant material.

Throughout the study, the environment in the chamber was maintained at 25°C and 75% relative humidity. Using these values, the concentration of tritium determined by the HT analyzer, and the concentration of tritium collected on the condensation coil, the ratio of HT to HTO in the gas phase was calculated to be:

Equation 1:
$$\frac{\text{HT (nCi/l)}}{\text{HTO (nCi/l)}} = 83$$

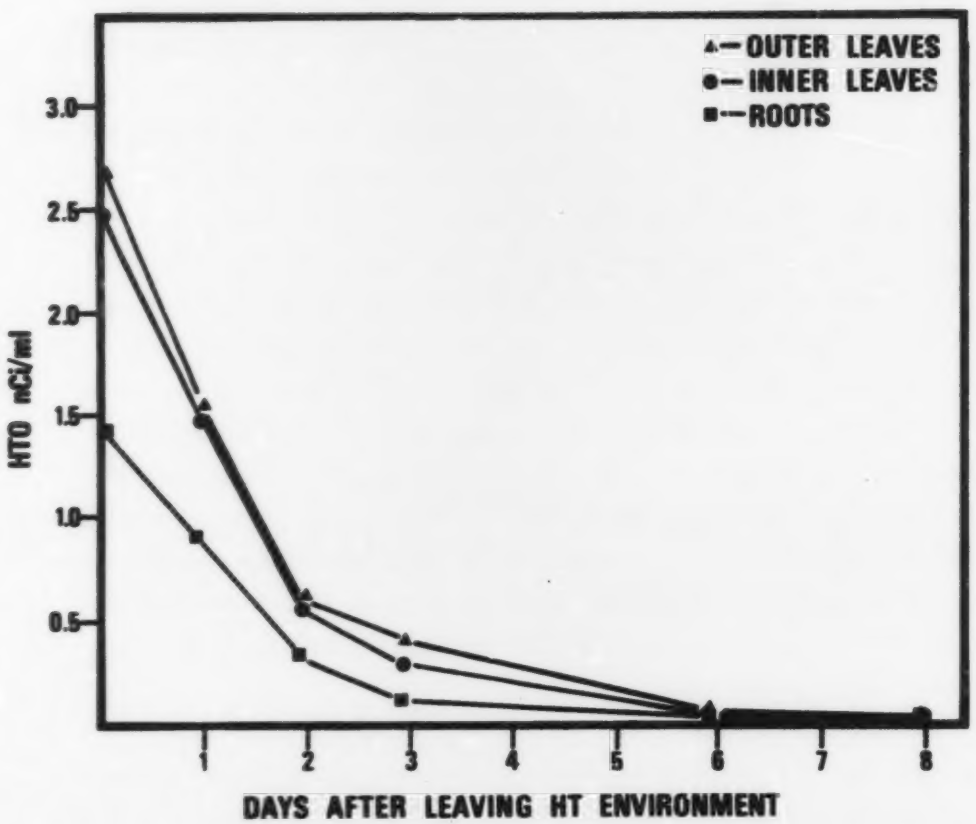

Figure 3. Tritium concentration [HTO] in the extractable plant water after removing lettuce plants from HT-contaminated environment.

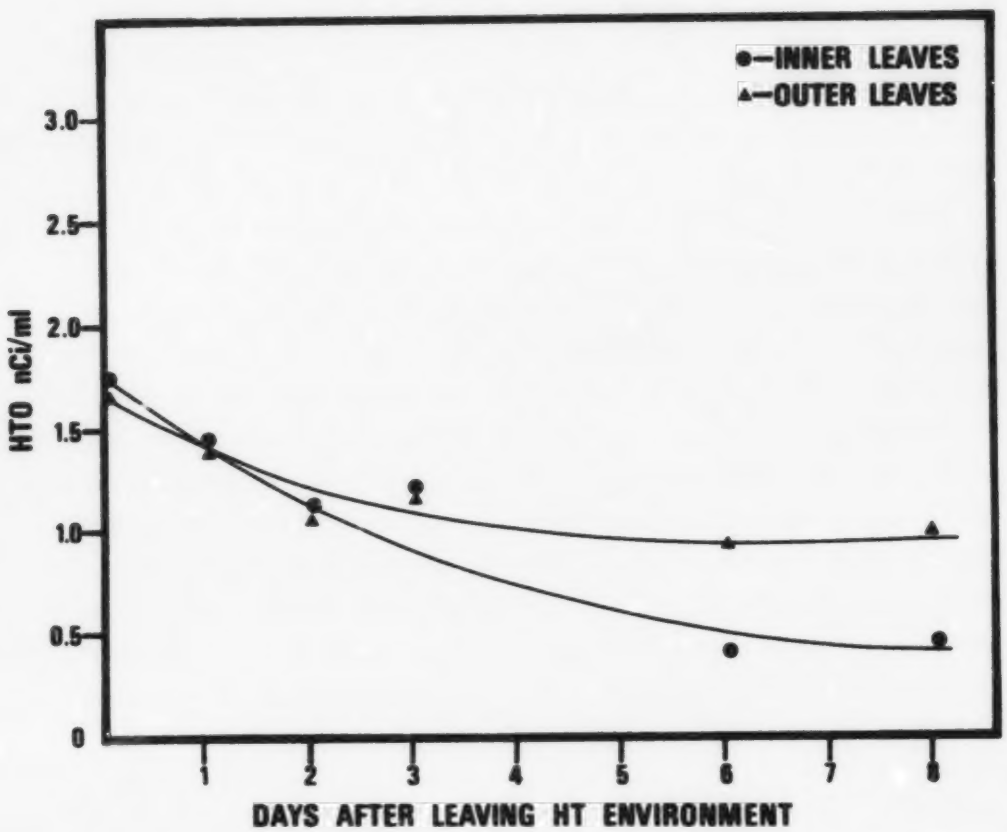

Figure 4. Organically incorporated tritium (combustion water) in lettuce plants after removal from HT contaminated environment.

That means there were approximately 83 times as many tritium atoms in the gaseous (HT) form than in the water vapor (HTO) form. By applying the gas law and using the specific activity of tritium (9.64×10^3 Ci/g), and assuming that the hydrogen concentration in the growth chamber air was 0.5 parts per million (Ehhalt et al. 1974) and the tritium concentration (HT) was 5 nCi/l, it was calculated that 2.58×10^5 atoms of hydrogen were present per atom of tritium. Similar calculations show that there were 6.46×10^{11} atoms of hydrogen per atom of tritium in the water vapor. Thus, on an atomic level there were approximately a million times as many tritium atoms per hydrogen atoms in the form of tritium gas than in the form of water vapor.

One hour after the initiation of HT fumigation, the first sample of transpired water was collected and analyzed for tritium. The elevated concentration of tritium in this sample indicated a rapid conversion of HT to HTO. Data were collected to compare the amount of HT injected into the chamber with the HTO lost through transpiration, leaching, and through incorporation into plant materials. In this budget, more than 95% of the tritium injected was accounted for in the form of HTO. The rest was presumably either lost when samples were removed, through leaks, or was not accounted for because of inaccurate measurement of water loss volumes. In control experiments in the absence of plants or soil, HT to HTO conversion rates were extremely slow and corresponded to the values reported by Eakins and Hutchinson (1973). No special effort was made in these experiments to correlate the tritium conversion rate with plant mass, area, metabolism, or soil microorganism activity, but from harvest data the fresh weight of the entire plant material was estimated. In Table 1, the HT injection rates and the HT to HTO conversion rates are listed. No valid correlation of conversion rate with leaf mass seems to exist, but a trend is apparent for conversion based on the number of pots. Also, despite the large

Table 1. RATES OF TRITIUM CONVERSION IN THE PRESENCE OF POTTED PLANTS

HT Treatment Period (days)	HT ⟶HTO Conversion Rate: per minute (nCi/min)	per pot (nCi/min/pot)	per g fresh wt. (nCi/min/g)
1 - 6	13.3	0.53	4.43×10^{-2}
6 - 11	13.7	0.72	0.38×10^{-2}
11 - 17	11.3	0.81	0.28×10^{-2}

gradient of elemental tritium to tritium oxide which is evident on a volume and atomic basis, and should favor chemical reactions, the rapid reaction rates observed only in the presence of potted plants suggest a facilitated oxidation or exchange. Whether the conversion reaction is a surface catalysis or an enzymatically facilitated reaction is unknown. The reverse reaction (production of H_2 from H_2O) is known to operate in lower life forms at rapid rates (Gest, 1954) and this suggests that a reaction occurring at the soil surface involving the soil microbiota is possible. Detailed studies regarding conversion rates and reaction sites are currently in progress.

For comparison, an experiment was conducted where plants were exposed for one week to tritiated water vapor. The concentration of tritium in the vapor was monitored and controlled by evaporating water of a known concentration. At the end of one week of exposure, leaves and roots were sampled and the water was extracted and analyzed for tritium. A comparison of these two experiments is best accomplished by examining the ratios of tritium concentrations in various segments of each experiment. Equation 2 describes the ratio of HTO concentrations found in plants and air when the plants were exposed to HT.

Equation 2: Plants exposed to 5 nCi/l of HT.

$$\frac{\text{HTO in leaves (nCi/ml)}}{\text{HTO in air (nCi/ml)}} = 0.84$$

Equation 3: Plants exposed to 115 nCi/ml of HTO in water vapor.

$$\frac{\text{HTO in leaves (nCi/ml)}}{\text{HTO in air (nCi/ml)}} = 0.34$$

A similar comparison is shown in Equation 3 when plants were exposed to HTO vapor. The higher ratio in Equation 2 probably indicates a more direct transfer of tritium when plants were exposed to HT. This may have been caused by a more available source (i.e. higher

HTO concentrations near the leaves than in the bulk air) or it may indicate that HT penetrated the plant and was converted in the substomatal cavity to HTO and subsequently absorbed from that side.

A portion of the tritium absorbed by the leaves was translocated in the phloem to the roots. The tritium concentration in the roots is dependent on its concentration in the leaves, on the transport rate in the phloem, and on the dilution rate caused by water transport through the xylem (McFarlane 1975). Equation 4 shows the ratio of tritium in the leaves and roots of plants exposed to HT, and Equation 5 shows a similar ratio of plants exposed to HTO vapor.

Equation 4: Plants exposed to 5 nCi/l air of HT

$$\frac{\text{HTO in leaves (nCi/ml)}}{\text{HTO in roots (nCi/ml)}} = 2.4$$

Equation 5: Plants exposed to 115 nCi/ml of HTO

$$\frac{\text{HTO in leaves (nCi/ml)}}{\text{HTO in roots (nCi/ml)}} = 75.6$$

The lower value in Equation 4 is thought to indicate a higher basipetal movement of tritiated water and labeled substrate. However, it should be remembered that soil contamination was not possible in the plants exposed to HTO vapor since a septum was placed between the foliar and subterranean portions of the plant.

After the third harvest, 10 pots previously unexposed were placed in the exposure chamber. Five contained 4-week-old lettuce plants and the others were filled with the potting mix (no plants). The automatic watering system was connected to all pots and after exposure to HT, the potting medium in each pot was mixed and a sample taken from each pot for analysis. The water was extracted and analyzed for tritium concentration.

On the basis of these data no significant differences were evident between the pots with and without plants. The large variation in tritium concentration in what were supposed to be replicate

samples was cause for concern. Since all pots were watered regularly it was presumed that repeated and thorough mixing would have resulted in the HTO concentration being rather uniform. The fact that this large variation occurred is an indication that the samples were stratified with respect to tritium and that a profile sampling should have been performed. Nevertheless, it is clear that the rooting media HTO was higher than the extractable root HTO (Figure 2). Thus the overall order of tritium contamination at equilibrium was soil > leaves > roots. Since the concentration in the roots was lower than in the soil, root uptake of tritiated water seems improbable. The tritium gradient from leaves to roots indicates that foliar absorption was the predominant plant contamination pathway. This apparent inconsistency is thought to have resulted from sampling the soils randomly and not taking a soil profile. If the soil surface was a reaction site for the conversion of HT to HTO, then a highly contaminated layer may have existed at the surface. Mixing this layer would have blended it with the layers lower in HTO concentration. This could account for the high and variable values in Table 2 and would offer an explanation for the apparent contradiction. Subsequent studies regarding soil contamination and stratification have proven this to be true and will be reported elsewhere (McFarlane and Batterman, 1975).

Six-inch petri dishes were placed in the chamber and 25 ml of uncontaminated water was placed within each dish. Samples were periodically removed, and the transfer resistance was calculated to be 3.4 cm/sec. Higher values would have been expected had the site of conversion been at the water surface. This additional bit of evidence supports the concept of a catalytically active surface perhaps on the leaves, or more probably at the surface of the soil.

Table 2. TRITIATED WATER EXTRACTED FROM POTTING MIXTURE AFTER EXPOSURE TO 5 nCi/l OF ELEMENTAL TRITIUM FOR 6 DAYS

A HTO extracted from mix (nCi/ml)	B HTO extracted from mix (nCi/ml)
2.05	0.87
1.28	1.11
1.94	1.22
2.77	0.99
1.22	1.99
$\bar{x} = 1.84 \pm 0.64$*	$\bar{x} = 1.24 \pm 0.44$*

A. Pots contained a potting mix of peat and vermiculite 1:1 (no plants).

B. Pots contained mix and one 4-week old lettuce plant growing in each pot.

* Standard deviation.

CONCLUSIONS

The presence of potted plants in the growth chamber facilitated the conversion of HT to HTO. Lettuce plants appeared to have been contaminated by the foliar absorption of tritiated water vapor. Although the site of the conversion reaction was not identified, high concentrations of HTO in the soil suggest that the soil or soil microorganisms may have been involved. Conversion half-times calculated from the injection rates in Table 1 range from 19 to 23 hours. It is clear from these values and from the observed elevated HTO concentration in the chamber water vapor only a short period after the start of the treatment that the release of elemental tritium into the environment may present a local contamination threat. If lettuce is representative of all plants, then guidelines for the release of elemental tritium should take into account the rapid conversion to HTO which occurs in the plant environment.

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